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Comparison of Use of Enzyme-Linked Immunosorbent Assay-Based Kits and PCR Amplification of rRNA Genes for Simultaneous Detection of *Entamoeba histolytica* and *E. dispar*

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A comparison of the use of three commercially available enzyme-linked immunosorbent assay-based kits and PCR amplification of rRNA genes to detect and differentiate *Entamoeba histolytica* from *E. dispar* was carried out. Only the Techlab kit did not cross-react with *E. dispar* antigens, but it was 100 times less sensitive than PCR in detection of and differentiation between the two types of *Entamoeba*.

A number of epidemiological studies and recent reports have shown that (i) all cases of invasive amebiasis are caused by *Entamoeba histolytica* and that the nonpathogenic type, *E. dispar*, is never detected in extraintestinal lesions (2, 11, 14, 21, 22, 26); (ii) persons infected with *E. histolytica* are occasionally asymptomatic (11); (iii) a significant percentage of individuals, especially in areas of endemicity, can be simultaneously infected with both *E. dispar* and *E. histolytica* (1, 19, 24); and (iv) persons found to be infected with *E. dispar* sometimes have intestinal symptoms and/or high titers of antiamebic antibodies (12, 13). Among the main reasons for the unclear picture of the epidemiology and frequently quoted world rates of amebiasis (25) are the uncertainties in the detection and differentiation of *E. histolytica* and *E. dispar*. The main purpose of the present study was to evaluate three commercially available diagnostic enzyme-linked immunosorbent assay (ELISA)-based kits for *E. histolytica* for their levels of sensitivity to detect and ability to differentiate between small amounts of *E. histolytica* and *E. dispar* and to compare them to an established PCR procedure which selectively amplifies the different rRNA genes of the two types of amebae (5, 19, 24).

ELISAs. Axenic cultures of *E. histolytica* HM-1:IMSS and xeniccultures of *E. dispar* SAW 1734R c1AR were grown in TYI-S-33 medium as previously described (6). Trophozoites were counted in a hematocytometer and lysed by freeze-thawing in phosphate-buffered saline containing a mixture of various protease inhibitors (leupeptin, 100 μg/ml; phenylmethylsulfonyl fluoride, 2 mM; iodoacetic acid, 5 mM; and phenanthrolene, 1 mM [all from Sigma Aldrich]).

Samples containing different amounts of trophozoite lysates were tested with three commercially available kits for detection of *E. histolytica* (a kit from Alexon Co., Sunnyvale, Calif.; a kit from Techlab, Blacksburg, Va.; and the Optimum S kit from Merlin Diagnostica, Bornheim, Germany) according to the manufacturers' instructions and with the reagents supplied in each kit by the manufacturer. Optical density was determined in an ELISA reader (Biotek Instruments). As an additional comparison, ELISAs were also performed with two monoclonal antibodies (MAbs) prepared in our laboratory. MAb 318-28 was prepared as previously described (4). MAb 116 was prepared by immunizing BALB/c mice according to the method of Galfre and Milstein (7) with a lipophosphoglycan (LPG) preparation, extracted and purified by hydrophobic and anion exchange from membranes of strain HM-1:IMSS trophozoites as described previously (18).

PCR. PCR was performed for 30 cycles and used as a template a DNA fraction that was solubilized after boiling a suspension of trophozoites (2×10^6/ml in phosphate-buffered saline) in a water bath (10 min) followed by centrifugation ($10{,}000 \times g$ for 10 min). Each of the two sets of selective oligonucleotide primers, for the *E. histolytica* and *E. dispar* small subunit rRNA genes, generated a product of 870 bp, as previously described (5, 15).

The results obtained with the different commercially available ELISA-based kits (Table 1) show that the most sensitive one is the Merlin Optimum S kit. It can easily detect antigen from 100 trophozoites of *E. histolytica*/well. On the other hand, this kit appears not to be sufficiently selective, as the antibodies against the serine-rich antigen, which are reportedly specific for *E. histolytica* (23), were found to cross-react, at higher concentrations (100 to 1,000 trophozoites/well), with antigens of *E. dispar*. This lack of selectivity can be a serious drawback, as it could lead to some false-positive *E. histolytica* results, especially since the majority of infected persons harbor *E. dispar* (2, 25, 26).

The Techlab kit, which uses MAbs against the Gal-specific lectin of *E. histolytica* strains (20, 21), was very selective and reacted, as reported, only with *E. histolytica*. However, its level of detection of amebic antigen, as also indicated by the manufacturer, was quite low and required approximately 1,000 trophozoites per well (8, 9). The high levels of *E. histolytica* antigen needed for detection suggest that this kit may not be able to detect low-level *E. histolytica* infections, especially in asymptomatic individuals in areas of endemicity who may be simultaneously infected with both types of parasites (1, 19, 24). The Alexon kit, which consists of anti-*E. histolytica* polyclonal antibodies which do not differentiate between *E. histolytica* and *E. dispar*, has fair detection sensitivity, and amebic antigens of either type can be detected at 100 trophozoites/well.

The levels of detection and selectivity observed with the two laboratory MAbs, MAb 318-28, which is specific for a lysine-rich surface antigen that is expressed on *E. dispar* strains (4), and MAb 116, which is specific for an LPG molecule present on the surfaces of virulent *E. histolytica* strains (17), were in the range of the manufactured kits (Table 1).

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TABLE 1. Comparison of different antibodies for detection of *E. histolytica* and *E. dispar* by ELISA

Antibody source or antibody	No. of trophozoites/well[a]	Detection of[b]:		Specificity
		E. histolytica HM-1:IMSS	*E. dispar* SAW 1734R clAR	
Techlab kit	10^4	1.058 ± 0.07	0.01	MAb against Gal-lectin of *E. histolytica*
	10^3	0.102 ± 0.01	ND	
	10^2	0.01	ND	
Alexon kit	10^4	0.874 ± 0.03	0.648 ± 0.05	Polyclonal antibodies against *E. histolytica*
	10^3	0.480 ± 0.05	0.350 ± 0.03	
	10^2	0.164 ± 0.03	0.085 ± 0.01	
Merlin Optimum S kit	10^4	1.764 ± 0.07	0.683 ± 0.02	MAb against serine-rich antigen of *E. histolytica*
	10^3	1.692 ± 0.06	0.149 ± 0.01	
	10^2	0.419 ± 0.02	ND	
Weizmann MAb 116	10^4	0.626 ± 0.03	0.05	MAb against LPG of virulent *E. histolytica*
	10^3	0.124 ± 0.02	ND	
	10^2	0.02	ND	
Weizmann MAb 318-28	10^3	0.02	0.776 ± 0.04	MAb against 30-kDa surface antigen of *E. dispar*
	10^2	0.01	0.410 ± 0.03	
	10	ND	0.03	

[a] Wells contained lysates.
[b] Values given in optical density units after color development of triplicate samples. ND, not detected.

The levels of detection observed with the various antibody-based systems were found to be >100-fold less sensitive than those that can be attained by PCR amplification of the rRNA genes of amebae. As shown in Fig. 1, PCR amplification can clearly detect the DNA from one single ameba in the sample. Moreover, the selectivity of PCR amplification of rRNA genes appears to be far superior to that of the antibodies, as it can detect one trophozoite of *E. histolytica* even in the presence of a 1,000-fold excess of *E. dispar* and vice versa. This is not surprising, since trophozoites have multiple copies of the rRNA genes (5, 10, 16).

Our results clearly indicate the advantages of PCR over ELISA-based kits in the ability to both detect and determine the type of amebae. Among the frequently mentioned arguments in favor of the use of ELISA versus PCR techniques are the convenience and lower price of ELISA-based kits, especially for the routine diagnostic laboratory in areas of endemicity. In view of the considerable improvements in the automation and simplification of PCR procedures for clinical sampling directly from stools (1, 3), as well as in the prices of equipment, reagents, and product detection systems that have been recently achieved, its comparison with ELISA has to be

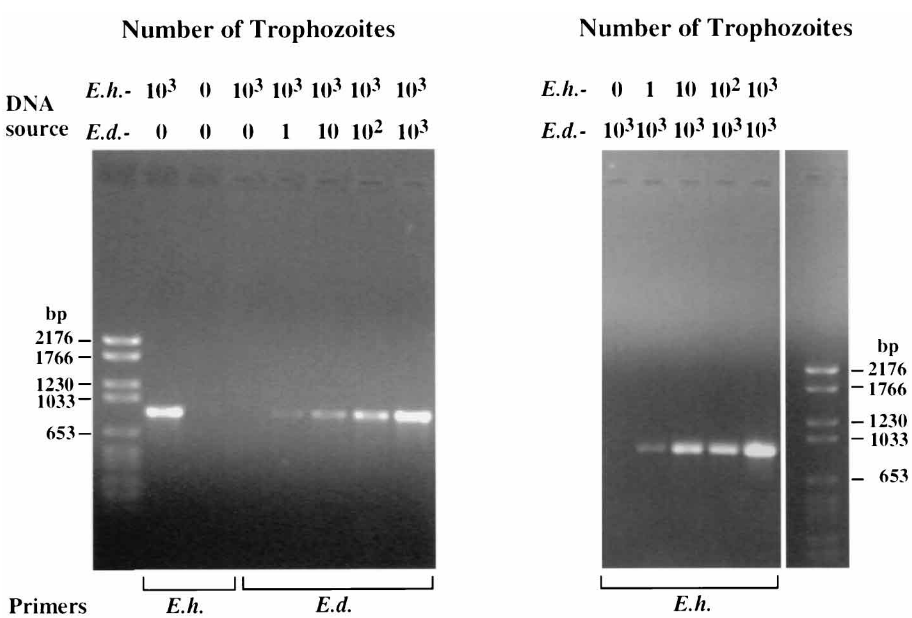

FIG. 1. Agarose gel separation of PCR-amplified products of the small subunit rRNA genes of *E. histolytica* (*E.h.*) and *E. dispar* (*E.d.*) (5, 15). DNA was prepared and diluted from a predetermined number of trophozoites (see Materials and Methods). (Left) DNA from one *E. dispar* trophozoite detected in the presence of 10^3 *E. histolytica* trophozoites; (right) DNA from one *E. histolytica* trophozoite detected in the presence of 10^3 *E. dispar* trophozoites.

carefully reevaluated by impartial experts. With respect to the epidemiology of amebiasis, it is important to accumulate data that are more accurate on the prevalence of *E. histolytica* in carriers and patients in various parts of the world. Based on the present comparative study on the available detection systems, this should be achieved preferably by PCR.

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